LC-MS/MS Determination of 3-HPMA (3-Hydroxypropylmercapturic Acid) in Human Urine

Curtis Sheldon[1], Ridha Nachi[1], Kirk Newland[1], Vinny Andaloro[1]
[1]MDS Pharma Services, Lincoln, NE

Purpose. To develop and validate a robust, rugged, sensitive and selective method for the determination of 3-HPMA in human urine. Acrolein is produced from many sources including automobile exhaust and cigarette smoke. 3-HPMA has been ~~identified (along with other mercapturic acids)~~ reported as a major biomarker for exposure to acrolein.

Method. A suitable control matrix was created by diluting urine 10-fold with NANOpure® water to dilute out endogenous levels. The 10-fold diluted control matrix was used to prepare the calibration curve for each batch. The samples were prepared using a solid phase mixed mode extraction column using deuterated 3-HPMA as internal standard. Following elution and evaporation of the eluent the extracts are reconstituted and injected on the LC-MS/MS. A Thermo Hypersil BioBasic AX, 50x4.6 mm, 5 µm column was used and 80:20 ACN: 50mM NH4Oac, pH4.5 as the mobile phase. The AB/MDS Sciex API 4000 using an ESI interface was used to analyze the extracts. Negative ions were monitored using multiple reaction monitoring. The run time was 4.5 min.

Results. The LC-MS/MS method for 3-HPMA was linear over the range of 35.0-5000 ng/mL. Quantitation in multiple lots of matrix at the LLOQ and ULOQ were acceptable after correction for endogenous levels. Acceptable intraday and interday accuracy (±7.0 % R.E.) and precision (≤7.5%) were attained over the range of the calibration curve. The mean extraction recovery for 3-HPMA and the internal standard were 80 and 85%, respectively. During analysis of study samples, an approximately 95% success rate was observed over approximately 20 batches.

Conclusion. The validated LC-MS/MS method is rugged, robust, sensitive and selective for the determination of 3-HPMA in human urine.

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